At the outset, there is no prima facie case of obviousness with respect to Daugherty et al. in view of Takaha et al.

Daugherty et al. describe protein refolding using β-cyclodextrin. See the Table at page 33963 of the reference. This reference fails to describe the cyclic saccharide cycloamylose having a polymerization degree of from 25-150 in combination with the specified detergents as claimed. In fact, β-cyclodextrin is the only cyclic saccharide described therein.

Takaha et al. describe producing cycloamylose with potato D-enzyme (see the Abstract). This reference fails to describe protein refolding with the cycloamylose. Rather, this reference describes the physical characterization of the cycloamylose product of the potato D-enzyme described therein.

These references, taken in combination, fail to suggest the claimed invention.

There is simply no suggestion in these references to substitute the cycloamylose described in Takaha et al. for the β-cyclodextrin described in Daugherty et al. Daugherty et al. do not describe any shortcomings of β-cyclodextrin which would motivate one to use a different cyclodextrin. Takaha et al. reports the physical characterization of the cycloamylose described therein. In the Official Action dated August 13, 2002, at page 5, the Examiner stated that Takaha et al. "discusses...the potential applications for cycloamylose." Significant by its absence is any mention of using the cycloamylose as a protein re-folding. Therefore, these references fail to suggest the claimed kits and methods.

In the Advisory Action, the Examiner has taken the position that a side-by-side comparison must be performed between the claimed cyclic saccharide cycloamylose and β-cyclodextrin in combination with the recited detergents. However, as discussed in the previous response, the present specification does present such data. That is to say, Table 1

shows the experimental result of refolding a protein, where Tween 40 was used as a detergent in combination with various cyclic saccharides. In comparing the refolding effect on denatured by each combination, cyclic saccharide cycloamylose CA(S) and CA(L) having a polymerization degree of 25 to 150 recovered 140% and 120% of activity, respectively. β-cyclodextrin of polymerization degree of 7 recovered 120% of activity as well, but this substance has a defect as described above. The result of γ-cyclodextrin having a degree of polymerization was 8% of the recovery and no refolding effect was observed. A similar experiment was carried out with a cyclic saccharide having a polymerization degree of 10 to 14, resulting in a recovery of only 4% of activity. Therefore, all the cyclic saccharides having various polymerization degrees do not always show a refolding effect. It is not expected from the cited references that the cyclic saccharide cycloamylose of the present invention, which has a much higher polymerization degree than the β-cyclodextrin of polymerization degree of 7, yields a far superior refolding effect.

In the Example 1 of the present application, citrate synthase (CS) is first denatured with guanidine hydrochloride and then refolded with a variety of artificial chaperones. See page 10 and page 13 of the specification. The results are presented in Table 1 at page 18 of the present specification.

The results of this experiment are set forth at page 19, first two paragraphs, of the specification which read as follows:

> Moreover, as to the change with the passage of time of the enzymatic activity, as apparent from Figs. 1 and 2, it has become clear that, in case of the artificial chaperon using CA(S) and CA(L) as the cyclic saccharide, the enzyme was refolded into the active form within as short as 2 hours after the addition of cycloamylose. That is, this shows that the artificial chaperon of the present invention has the ability of refolding the denatured protein in an unfolded state correctly within a short time.

> On the other hand, in case of β-CD, only from about 30 to 40% of the enzymatic activity was recovered 2 hours after the addition of the β-CD, and it took more than overnight to recover 100% of the enzymatic activity.
>
> Therefore, it has become clear that cycloamylose is more preferable agent used as the artificial chaperon of the present invention.

Thus, the present specification provides the very side-by-side comparative data that the Examiner is looking for. As described at page 17 of the specification, Figure 1 of the present application presents the time course of the recovery of enzymatic activity using different cyclic saccharides and Tween 40, and Figure 2 presents similar data for Tween 60. As stated in the passage from the specification described above, using the claimed re-folding agent the enzyme was refolded into the native form within as short a time period as 2 hours. In contrast, with β-cyclodextrin, only about 30 to 40% of the enzymatic activity was recovered in 2 hours, and it took more than an overnight incubation to recover 100% of the enzymatic activity.

The cited references fail to suggest these striking results.

Daugherty et al. describe protein refolding using β-cyclodextrin. This reference fails to describe the cyclic saccharide cycloamylose having a polymerization degree of from 25-150 in combination with the specified detergents as claimed.

Takaha et al. describe producing cycloamylose with potato D-enzyme (see the Abstract). Nothing in this reference suggests using the a cyclic saccharide cycloamylose having a polymerization degree of from 25-150 in combination with the specified detergents for refolding proteins. Rather, this reference describes the physical characterization of the cycloamylose product of the potato D-enzyme described therein. Thus, this reference contains no teaching or description of protein refolding whatsoever.

One with Daugherty et al. and Takaha et al. in hand would not have predicted that a cyclic saccharide cycloamylose having a polymerization degree of from 25-150 would be dramatically more effective as compared to β-cyclodextrin for refolding proteins as demonstrated by the data presented in the present specification. Daugherty et al. describe protein refolding using β-cyclodextrin and is silent with respect to a cyclic saccharide cycloamylose having a polymerization degree of from 25-150. Takaha et al. describe a a cyclic saccharide cycloamylose within the scope of the claims, but is silent with respect to refolding proteins. Given the teachings of the references, one would simply not be led to expect the striking results set forth in the present specification.

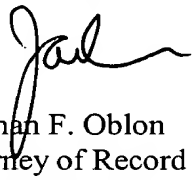
Based on the foregoing, the combination of Daugherty et al. and Takaha et al. fails to suggest the claimed kits and methods. Therefore, the claims are not obvious over those references. Accordingly, withdrawal of this ground of rejection is respectfully requested.

The rejection under 35 U.S.C. §112, second paragraph, is believed to be obviated by the amendment submitted above. Claims 32, 36, 40, and 47 have been amended to recite a Markush format. Accordingly, withdrawal of this ground of rejection is respectfully requested.

Applicants submit that the present application is in condition for allowance. Early notice to this effect is earnestly solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.



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Amendment Filed On: HEREWITH

IN THE CLAIMS

Please amend the claims as follows.

--32. (Amended) The kit of Claim 31, wherein the polyoxyethylenic detergent is selected from the group consisting of [a] polyoxyethylenesorbitan ester, polyoxyethylenedodecyl ether, polyoxyethyleneheptamethylhexyl ether, polyoxyethyleneisooctylphenyl ether, polyoxyethylenenonylphenyl ether, polyoxyethylene fatty acid ester and [or] sucrose fatty acid ester.

36. (Amended) The kit of Claim 35, wherein the ionic detergent is selected from the group consisting of cetyltrimethylammonium bromide, sodium dodecyl sulfate, sodium deoxycholate, 3-[(3-colamidopropyl)dimethylammonio]-1-propanesulfonic acid, hexadecyltrimethylammonium bromide and [or] myristylsulfobetaine.

40. (Amended) The method of Claim 39, wherein the polyoxyethylenic detergent is selected from the group consisting of [a] polyoxyethylenesorbitan ester, polyoxyethylenedodecyl ether, polyoxyethyleneheptamethylhexyl ether, polyoxyethyleneisooctylphenyl ether, polyoxyethylenenonylphenyl ether, polyoxyethylene fatty acid ester and [or] sucrose fatty acid ester.

47. (Amended) The method of Claim 46, wherein the ionic detergent is selected from the group consisting of cetyltrimethylammonium bromide, sodium dodecyl sulfate, sodium deoxycholate, 3-[(3-colamidopropyl)dimethylammonio]-1-propanesulfonic acid, hexadecyltrimethylammonium bromide and [or] myristylsulfobetaine.--